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An Alternative Strategy for Fabrication of Robust and Flexible Digital Microfluidic Devices

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Abstract
We present a new strategy for the fabrication of digital microfluidic (DMF) devices by research groups without access to well-equipped facilities. Compared to previously developed methods, it increases considerably device yield, and yet simplifies fabrication and enables flexible operation.

To this end, we use large (260 and 400 μm) interelectrode gaps to optimize substrate fabrication, breaking the established constraint that gap dimensions must be small (usually < 150 μm) for digital microfluidics. In fact, we show that interelectrode gaps are not limiting features for DMF performance, and may be quite large (> 1 mm). Smooth droplet actuation is ensured by films of perfluoroalkoxy (PFA), which do not require additional coatings; films can be instantly replaced, without the necessity of annealing. Finally, our tests indicate that DMF devices can operate with large distances between plates and droplet volumes (up to 2 mm and 60 μL, respectively), which implies in a flexibility in device operation not observed before.

Devices can move, split, and dispense from a reservoir, including droplets containing cells (the ciliated protozoa *Tetrahymena thermophila*). Altogether, we believe that the new strategy presented here will help expand the breadth of DMF applications.

Keywords
digital microfluidics, large-gap electrodes, alternative devices

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INTRODUCTION

The advantages associated with low consumption of reagents and fast analysis time have made microfluidics attractive for "lab-on-a-chip" applications (Chen & Drew, 2008; Fair, 2007; Freire & Wheeler, 2006). In particular, digital microfluidics (DMF) is a technique for manipulation of droplets on hydrophobic surfaces, by an electric potential applied to an array of electrodes (Cho *et al.*, 2003; Lee *et al.*, 2002; Malic *et al.*, 2009; Schwartz *et al.*, 2004; Velev *et al.*, 2003; Washizu, 1998). DMF enables automation and control of droplets unparalleled by any other technique of fluid transport (M. Abdelgawad *et al.*, 2008; Böhringer, 2006; Fair, 2007), and droplets containing a wide variety of reagents (Chatterjee *et al.*, 2006) can be manipulated simultaneously, without the need for pumps, tubes, or valves.

Despite the enthusiasm, the access to DMF devices continues to be a barrier for researchers without access to well-equipped facilities (i.e., mostly clean-rooms) (Barbulovic-Nad *et al.*, 2008; Gong & Kim, 2008; Vijay Srinivasan *et al.*, 2004). Following the trend observed in channel microfluidics (do Lago *et al.*, 2004; Grimes *et al.*, 2008; C. W. T. Yang *et al.*, 2010), other fabrication methods have been developed for DMF devices (Abdelgawad & Wheeler, 2008), including prototyping on copper laminates using commercial printers (Abdelgawad & Wheeler, 2007); however, our tests revealed that this promising method required further development, as discussed below.

In fact, during our fabrication efforts, printed copper substrates often had short-circuited electrodes, rendering the devices inoperable. In addition, to form the surface for droplet actuation, substrates had to be coated with thick layers of dielectric (9 or 35 µm), which we found time consuming and difficult to perform, leading to low device yield; although the use of polyethylene films (Abdelgawad & Wheeler, 2008; Gorbatsova *et al.*, 2009) or parafilm (Jebrail *et al.*, 2010) has been suggested, these materials seem to require additional coatings and/or annealing for optimal droplet actuation, and were not used here.

We adopted procedures to address these problems. Here, a film of perfluoroalkoxy (PFA), which served as a dielectric and hydrophobic layer, was attached to the top of the electrode array, avoiding droplet trapping between electrodes. No additional coatings were necessary. If required, films could be replaced instantly, without the necessity of annealing; this flexibility is a desirable characteristic, since failures in DMF devices are often related to the surfaces for droplet actuation (e.g., caused by non-specific adsorption). In addition, PFA films are autoclavable, and may be a suitable alternative for assays requiring sterile conditions.

Contrasting with the current state of knowledge (Abdelgawad & Wheeler, 2008), we demonstrate, for the first time, that interelectrode gaps (usually smaller than 150 µm (Abdelgawad & Wheeler, 2007)) are not limiting features for droplet operations (i.e., moving, splitting, and dispensing) in DMF devices, and can be quite large (> 1 mm). Therefore, miniaturization is not required, which may spark new methods of fabrication, contributing to increase the accessibility of various research groups to DMF. Although copper substrates have been used before, it is particularly important to mention that the use of large interelectrode gaps increased significantly the number of successful printed copper substrates – which are robust, and can be used for several times. We have chosen gaps of 260 and 400 µm, which allowed smooth motion of droplets with volumes from less than 1 up to 60 µL,

using interdigitated electrodes. These "alternative devices" could move, split, and dispense droplets from a reservoir. During the last year, we have been using these DMF devices on a daily basis in the laboratory.

As applications continue to expand, DMF has been touted as an important tool for cell-based analysis (Barbulovic-Nad *et al.*, 2010; Shah *et al.*, 2009). Here, we introduce preliminary studies of *Tetrahymena thermophila* cells (strain SB 255) on the alternative DMF devices. These ciliated protozoa swim in fresh water (speed ~0.4 mm/s (Koutna *et al.*, 2004)), and have been a source for analysis and purification of cilia, microtubules, and dyneins. Cells were successfully actuated, remaining intact, and possibly with no harmful effects, an indication that the relatively high voltages required for device operation do not limit biological applications.

MATERIALS AND METHODS

Silicone oil (DMS-T01) was from Gelest Inc. (Morrisville, PA). Food coloring dye (blue) was from McCormick & Co., Inc. (Hunt Valley, MD). Copper etchant (CE-100) was from Transene Company, Inc. (Danvers, MA). Fluorinert FC-40 was from Sigma-Aldrich (St. Louis, MO), and Teflon-AF 1600 from DuPont (Wilmington, DE). Silicone rubber 732 was from Dow Corning (Midland, MI). Perfluoroalkoxy (trade name) (PFA) film (30 µm thick), was from McMaster-Carr (Robbinsville, NJ). Indium tin oxide (ITO)-coated glass slides were from Delta Technologies, Limited (Stillwater, MN). Pyralux double-sided copper-clad laminate AP7163E, and single-sided copper-clad laminate composite LF9110 were from DuPont Flexible Circuit Materials (Research Triangle Park, NC). The thickness of the copper layer was 9 and 35 µm, respectively, and will be designated as thin and thick substrates. For modified Neff medium (0.25% proteose peptone, 0.25% yeast extract, 0.55% glucose and 33 µM $FeCl_3$), all reagents were from Sigma-Aldrich (St. Louis, MO).

Tetrahymena thermophila cells (strain SB 255) were from the *Tetrahymena* stock center at Cornell University (Ithaca, NY). Cells were cultured in modified Neff medium at 20 °C (Cassidy-Hanley *et al.*, 1997). The concentration was approximately 10^5 cells/mL.

Patterns for digital microfluidic devices were printed on copper laminates using a laser Xerox Phaser 6360N printer (Xerox Corporation, Norwalk, CT), and all the unprotected copper was etched in CE-100, according to the procedure previously developed (Mohamed Abdelgawad *et al.*, 2008) – however, for other applications. Etched laminates were then attached to regular glass slides (75 x 25 mm) using double-sided tape. Thin and thick substrate designs (1) were linear, composed by 8 or 10 interdigitated electrodes, or (2) included vertical motion and reservoir for dispensing. Interelectrode gaps were 260 and 400 µm. No masks were required for fabrication.

Previously, it has been suggested that PDMS could be used as the dielectric layer, and subsequently coated with Teflon-AF to render the surface hydrophobic (Abdelgawad & Wheeler, 2007). However, our tests revealed reduced device reliability when using this procedure, particularly on thick laminates (35 µm copper layer thickness); droplets were almost always trapped between electrodes.

Therefore, we adopted a new procedure to optimize fabrication, using a film of perfluoroalkoxy (PFA) to form the dielectric layer (30 µm thick). The contact angle for this fluoropolymer is 110º, and no additional coating with Teflon AF was necessary. The film was attached to the substrate using silicone rubber (732), which was applied using a spatula, and the excess

carefully removed; a strip of PFA film was then placed on the surface and evenly pressed, using a clean glass slide. The adhesive was left to cure overnight. The film was bridging the gap between electrodes, preventing droplet trapping; this was a critical feature, especially for devices formed on thick electrodes.

Adhesion was strong enough to firmly hold the film in place for device handling and operation; however, the PFA film could be removed and replaced several times, with the same adhesive layer. Removable films have been regarded as an effective way to introduce pre-loaded reagents onto DMF devices (H. Yang *et al.*, 2009), and PFA may be a suitable, low cost option. Here, no thermal treatment (i.e., annealing), which may be detrimental to the activity of reagents, was required for the attachment of films to substrates.

Although droplets can be successfully moved using a single-plate configuration (M. Abdelgawad *et al.*, 2008), here, all DMF devices were enclosed, to enable droplet splitting and dispensing (Cho *et al.*, 2003); however, the devices could move droplets up to 60 µL, which we believe was the largest volume transported in enclosed devices (droplet volumes are usually less than 10 µL). The top of devices were formed by ITO-coated slides spin coated (spinner WS-400B-NPP Laurell Technologies, North Wales, PA) with Teflon-AF (Teflon-AF resin in Fluorinert FC-40, 1.5:100 (w/w), 2000 rpm, for 1 minute), and then baked on a hot plate (160 ºC, 10 minutes). A manual actuator was used to set the distance between top and bottom plates; however, pieces of double-sided tape (95 µm thick each) were also used when convenient.

Droplets were actuated by applying AC voltages (15 kHz, 500 – 660 V_{RMS}) to electrodes, and were obtained by a signal generator (33220A, Agilent Technologies, Santa Clara, CA), connected to a high voltage amplifier (PZD700, Trek, Inc., Medina, NY). Device operation was monitored by a Hitachi CCD camera and imaging system (VZM 200i, Edmund Optics, Barrington, NJ).

These higher operation voltages were due to the use of thicker films (30 µm thick PFA film), and contrasts with the lower voltages (~120 V_{RMS}) used in clean-room based devices (Barbulovic-Nad *et al.*, 2008). However, higher voltages allowed successful device operation, and, currently, there are no indications of harm to actuated cells.

Droplets were actuated in three different forms: Manual, by direct application of electric potentials to the electrodes; semi-automatic, by an in-house fabricated relay interface controlled by LabView (National Instruments, Austin, TX), which allowed simultaneous, handsfree, application of voltage to electrodes; and automatic, with timed application of voltages controlled by an in-house developed code. In both cases, the relays provided a reliable control of droplet operations (i.e., moving, splitting, and dispensing).

RESULTS AND DISCUSSION

Large interelectrode gaps were not impediment to droplet actuation

To test the conditions for droplet actuation, a simple set-up was designed, composed by a PFA film extended between two aluminum electrodes. Droplets of DI water were positioned in the gap. The interelectrode gap was set quite large (0.5 cm), enabling, however, droplet motion (not shown), and split (Fig. 1, (a) and (b)).

Therefore, contrasting with the current state of knowledge (Abdelgawad & Wheeler, 2008), interelectrode gaps

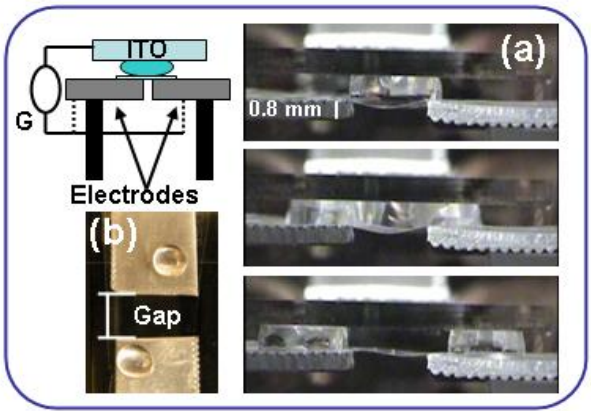


Figure 1: Top left: Schematic view of the set-up to test droplet actuation. Droplets were confined between the ITO slide (coated with Teflon AF) and the PFA film, and could be moved and split by the application of voltage (660 V, 15 kHz, generator G) to two aluminum electrodes (~1 cm wide and 0.8 mm thick). (a) Sequence (top to down) showing a droplet split (DI water, 50 µL) across an interelectrode gap of ~0.5 cm. The top view of droplets after splitting can be seen in (b).

(usually smaller than 150 µm), are not limiting features for droplet operations in DMF devices, and can be quite large – in these tests, 33 X larger. This choice is not unique; for a given gap, the distance between plates can be adjusted, to optimize actuation of droplets with different volumes.

Here, we have chosen gaps of 260 and 400 µm, which allowed smooth motion of droplets with volumes from less than 1 up to 60 µL, using interdigitated electrodes. To our knowledge, these are the largest gaps between electrodes described in the literature, confirming that device performance (i.e., the capability of moving, splitting, and dispensing droplets) is not tied to small interelectrode gaps.

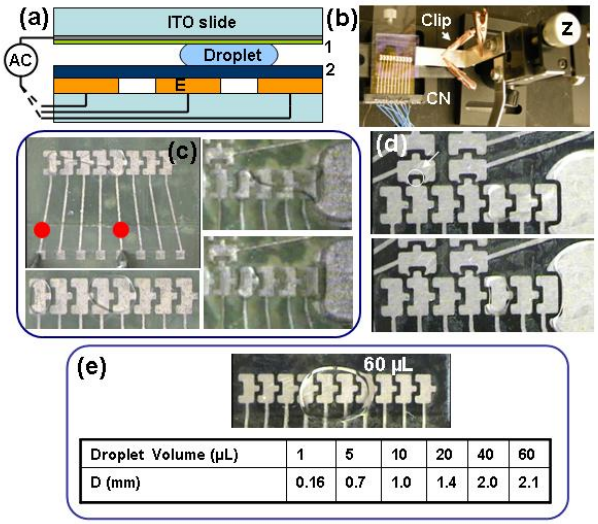
In addition, since the PFA film was extended across electrodes (Fig. 1(a)), electrode thickness was not a limitation for device operation. The PFA film provided a smooth surface for droplet movement, even with considerably thick (0.8 mm) aluminum electrodes. Therefore, the simplest device for droplet actuation could be formed by two pieces of metal (of any thickness), close together (millimetric range, as in Fig. 1), and covered with a PFA film.

Droplet operations

The performance of DMF devices was analyzed, using an enclosed configuration (Fig. 2 (a)); the distance between plates was controlled by a mechanical actuator (Z, in Fig. 2 (b)). Thin (9 µm) and thick (35 µm copper) substrates supported droplet splitting and dispensing from a reservoir (Fig. 2 (c) and (d)), and thick substrates, which were cheaper and easier to handle, were used for the remainder of the experiments.

Droplets of DI water with volumes ranging from less than 1 µL to 60 µL could be moved, with distance between plates adjusted from 0.16 mm to 2 mm (see part (e)) to optimize motion; this is a novel aspect, not mentioned before in the DMF literature, and may be convenient for the introduction of probes or sensors for different purposes in the devices, and in assays involving diluted analytes that could benefit from large droplet volumes. The operations were controlled manually (Fig. 2 (c)), or semi-automatically ((d) and (e)). Although droplet-droplet repulsion (Vallet *et al.*, 1996) was observed, droplet merging occurred after a few seconds, in all experiments.

The direct contact of cells and media with the PFA film rendered the surface hydrophilic, impeding droplet transport. This phenomenon, non-specific adsorption, is a challenge in microfluidics, and is also observed in DMF devices (including the ones fabricated in clean-rooms). A common approach (V. Srinivasan *et al.*, 2003) is the use of silicone oil, which was adopted here; the oil isolates the droplet from the film, and enabled all droplet operations on thick substrates (Fig. 3).



Droplet Volume (µL)	1	5	10	20	40	60
D (mm)	0.16	0.7	1.0	1.4	2.0	2.1

Figure 2: (a) Schematic view of the DMF devices. ITO slides were coated with Teflon AF (1) and used to confine the droplet on the top of PFA film (2). Voltages were applied between the ITO slide and the electrodes (E) for droplet operations. (b) View of the experimental set-up. The clips were used to fix and ground the ITO slide; the connector (CN) was used to apply voltages in the semi-automatic and automatic configuration. The distance between plates (~ 0.4 mm) was adjusted by a manual positioner (Z). (c) Droplet splitting (left, 8 µL total volume) and dispensing (right; reservoir, 20 µL, and individual droplet ~ 2µL) on thin substrates. Voltages were applied manually (e.g., as indicated by dots). (d) Dispensing (same volumes as above), in devices formed on thick substrates, using a semi-automatic control of voltages. In all devices, interelectrode gaps were 400 µm; exceptions were the 260 µm gaps indicated by the white circle. (e) Distance between plates, D, could be varied, to move droplets of DI water up to 60 µL using a semi-automatic control of voltages.

Application: Operations with droplets containing cells

Advantages of DMF include the automated manipulation of droplets, the capacity to effectively stop flow without movable parts, and minimal (possibly negligible) sample heating (Fair, 2007). DMF is becoming an attractive technique for diverse applications (M. Abdelgawad *et al.*, 2008; Freire & Wheeler, 2006), including studies of cells and proteins (Barbulovic-

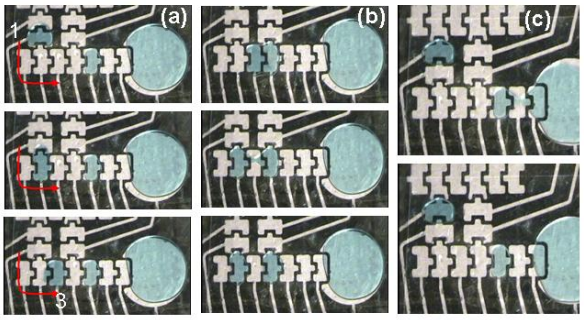


Figure 3: Operations with DI water droplets (spiked with food colorant), and using thick substrates with a thin layer of silicone oil. (a) Motion, from positions 1 to 3. (b) Droplet splitting, and (c) dispensing. The distance between plates was 400 µm (+/- 10%); the volume in the reservoir was 20 µL, and for the smaller droplets, approximately 2 µL.

Nad *et al.*, 2010; Barbulovic-Nad *et al.*, 2008; Jebrail *et al.*, 2009; Shah *et al.*, 2009).

We tested the capacity of alternative DMF devices to actuate droplets containing *Tetrahymena thermophila* (SB 255) cells in media, and analyzed cell survival. The reason for this choice is that, in future applications, we intend to use DMF to provide accurate time delivery of reagents to study ciliary motion, and these cells provide a large number of cilia, with short generation time.

Here, the volume of individual droplets was chosen to be larger than 2 µL for optimal off-chip manipulation, since smaller volumes tended to stick to pipette tips during handling. Silicone oil was applied to the PFA film to reduce non-specific adsorption, enabling droplet motion.

As preliminary tests for cell survival, 5 µL droplets containing SB 255 cells in media were moved 40 times on linear devices, and compared to non-actuated droplets; cell counting (using a hemocytometer) showed no significant difference in the number of live (motile) cells. For further tests, easily removable films were quite convenient - PFA films were detached from the devices (after 150 droplet transfers), and inspection on a microscope revealed no dead cells, inside

the droplet or along the actuation path. In all cases, cell shape and motility seemed unaffected after motion, indicating that devices were compatible with cell actuation, with no harm to cells.

Therefore, the relatively high voltages (500 – 660 V_{RMS}) required for device operation seem not detrimental to cell vitality. This corroborates previous results showing that the voltage across the droplet in a DMF device is an insignificant fraction of the total applied voltage (Barbulovic-Nad *et al.*, 2008), indicating very little, if any, effects on cells.

Devices supported motion, splitting, and, for the first time using printed copper laminates, dispensing of droplets containing cells (*Tetrahymena thermophila*) (Fig. 4). No dilution of the media constituents was required.

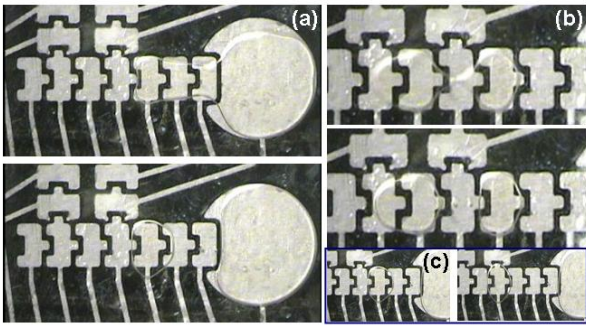


Figure 4: Dispensing (a), splitting (b), and moving droplets (c) containing *Tetrahymena thermophila* cells in media, on oil, and using thick substrates. Distance between plates and droplet volumes as in Fig. 3.

Comparison to other fabrication methods

The yield and performance of the alternative devices was compared to the clean-room fabricated ones (Fair, 2007; Watson *et al.*, 2006) (Table I). Although fabrication using printed copper laminates has been reported (Abdelgawad & Wheeler, 2007), there are no quantitative studies regarding droplet speed, number of droplet transfers, and number of successful fabricated substrates.

Therefore, for comparison, we also printed substrates with small gaps (200 µm as reported before (Abdelgawad & Wheeler, 2007). We verified that the number of successful printed substrates was limited; in fact, 47% of them had at least one pair of short-circuited electrodes. However, when using large interelectrode gaps (260 and 400 µm), the great majority of printed substrates were operational, with no short-circuit (measured using an ohmmeter) between pairs of electrodes; this was comparable to the yield of conventional fabrication in clean-rooms (Watson *et al.*, 2006).

To characterize device performance, droplets containing cells in media (4 to 6 µL), on oil, were automatically actuated (time of residence on each electrode 0.2-0.4 s). Devices could perform at least 20,000 droplet transfers, as long as evaporation was compensated for. No damage of films was observed after periods of 1.5 hour of intermittent operation; in fact, once attached and without wrinkles, > 90% of PFA films enabled at least 40 droplet transfers per film. Annealing was not required for film adhesion, which could be replaced instantly if damaged or contaminated (see supplementary movie). The maximum actuation speed was ~10 cm/s (4 µL DI water droplet, on oil). Therefore, the performance was close to the observed in clean-room devices (Fair, 2007) (see Table I).

CONCLUSION

We present a novel and easy strategy for fabrication, which increases considerably the number of successful DMF devices, representing a significant improvement when compared to previous methods – it leads to easier fabrication/operation of more robust devices than what has been done before.

	DMF devices with printed copper substrates		clean-room
	alternative (this work)	previous method*	
successful substrates	93% (large gap, N=15)	47% (small gap, N=15)	97%[b]
number of droplet transfers	20,000 (N=3)[a]	-	25,000
maximum droplet speed	10 cm/s (N=3)	-	25 cm/s

Table I: Comparison of yield and performance of devices fabricated here and by other authors. The use of large interelectrode gaps increases significantly the number of successful printed substrates, and the strategy developed here enables the fabrication of DMF devices with performance comparable to their clean-room counterparts in number of operations and droplet speed. [a]As long as evaporation is compensated for. [b]Actually, this number refers to the percentage of successful *electrodes* in devices (Watson *et al.*, 2006); here, we compare with the number of successful *devices*, with all electrodes operational. We actually printed and tested substrates with small gaps (~200 μm), and 47% of them had at least one pair of non-operational electrodes (i.e., short-circuited – see text). N represents the number of tested devices; no available data in the literature is indicated by "-".*(Abdelgawad & Wheeler, 2007).

It is developed particularly for researchers without access to well-equipped facilities. Altogether, we believe that this strategy broadens the horizons of device fabrication and operation, taking advantage of the following: miniaturized interelectrode gaps are not required for device operation; films for droplet actuation can be promptly changed, with reusable substrates; and the distance between top and bottom plates can be large (up to 2 mm) to move unconventionally large droplets (up to 60 μL). This flexibility may be convenient for the introduction of probes and sensors for different purposes in the devices, and in assays involving dilute analytes which could benefit from larger droplet volumes.

Cells can be successfully actuated on the devices, and DMF will be used to characterize aspects of ciliary biophysics in future applications. Of particular relevance is that the higher actuation voltages (when compared to conventional devices) are not harmful to cells, which makes "alternative" DMF devices promising for bioanalytical applications in general, contributing to increase the accessibility of various research groups to DMF.

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